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FOREWORD

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Andrew D. Seidman, M.D.

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PI - Signature Date

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EGF Receptor Monoclonal Antibodies and Chemotherapy in Breast Cancer Therapy

Technical Objectives

This project is based on the bioactivity of monoclonal antibodies (Mabs) against the epidermal growth factor receptor (EGFR) that we have produced (1). The human:murine chimeric version of Mab 225 (HC Mab 225) has been produced by ImClone Systems and has been made available to us in quantities adequate for a series of clinical trials. These Mabs inhibit the growth of tumors expressing the receptor and synergize with either doxorubicin or paclitaxel against well-established tumor xenografts (2-5). Preliminary clinical trials with murine anti-EGFR Mabs conducted by our group have shown that their administration is safe and that plasma levels of Mab sufficient to saturate receptors can be achieved (6,7). This project is to conduct a series of clinical trials to determine the safety, feasibility, and noncomparative efficacy of chemotherapy plus Mab in the treatment of patients with metastatic breast cancer who have not received extensive prior chemotherapy for their advanced disease. A parallel series of investigations in animal models (J. Mendelsohn, Principal Investigator) is being conducted to define other promising drugs, doses, and schedules, and to study mechanisms of action in these systems.

Experimental Approach

We proposed to conduct two phase I/II studies in patients with metastatic breast carcinoma with tumors that express high levels of EGFR. The first study was to be the combination of doxorubicin and anti-EGFR MABs. However, after thorough review of the preclinical data, we elected to first proceed with the study of paclitaxel and anti-EGFR Mabs. This decision was also based on considerations of patient availability, since doxorubicin is now widely used in the adjuvant setting. In the conduct of our trial we have carefully monitored for safety, pharmacokinetics, and clinical response. Although we still plan to biopsy accessible tumors before and after exposure to therapy to evaluate for EGFR regulation, phosphorylation, and for apoptosis, issues of toxicity, response, and optimal schedule need to be successfully addressed prior to initiating that phase of the investigation. Skin biopsies have been obtained in three patients to assess the histology of toxic reactions.

Progress To Date

The construction of a feasible phase I/II trial required the determination of the safety and pharmacokinetics of multiple administrations of the drug HC Mab 225. We therefore performed an open-label dose-escalation study of four weekly infusion at the dose levels of 5 (one patient completing twelve weeks of therapy), 20 (two patients), 50 (one patient), and 100 mg/m2 (three patients) per week in patients with histologically documented advanced tumors over-expressing EGFR by immunohistochemistry. (A total of twelve patients were enrolled at MSKCC, with five comparable patients accrued at other centers). The median age of our patients was 60 years, and

several tumor types were represented, including breast cancer. Only one patient experienced grade 3 toxicity, an episode of "aseptic meningitis" perhaps unrelated to drug administration. There was one grade 2 allergic reaction. All other toxicities were grade 1. These included: acneiform rash (3 episodes), fatigue (2), hot flashes (1), anorexia (1), chills (1), flu-like symptoms (1), thrombocytopenia (1), stomatitis (1), elevations of alkaline phosphatase (1), and creatinine (1). Pharmacokinetic values were assessed by the BIAcore (suface plasmon resonance) assay on serum samples drawn at1/24, 3/24, 6/24, 1,2,5,8,15,22,26,and 28 days post infusion. We sought to obtain a serum level of at least 20 nM, since preclinical evidence suggested that this level would be sufficient to occupy a high proportion of receptors in target tissues. (The notion of "saturation of receptors" does not apply since EGFR is widely distributed in normal organs). When 50 mg/m2 was given, the mean concentration of drug was greater than 20 nM for more than one day. At 100 mg/m2 the mean concentration of drug was greater than 20 nM for more than a week, allowing for drug accumulation. Saturation of clearance was not seen. Hence we became confident that a trial employing weekly administrations of 100 mg/m2 doses of drug would be adequate to elicit the desired biological effects.

Our phase I/II trial of the combination of HC Mab 225 and paclitaxel was designed to accrue patients with histologically documented metastatic breast cancer, regardless of immunophenotypic expression of EGFR. A copy of the clinical protocol is appended. To be eligible for study, a patient must have bidimensionally measurable disease, normal hematologic and organ function, a Karnofsky Performance Score of greater than 50%, no prior taxane (paclitaxel or docetaxel), and not more than one prior chemotherapy regimen for metastatic disease. The study was designed to accrue three patients each at the following initial and subsequent doses in mg/m2 of HC Mab 225: 50/50, 100/100, 200/100, 400/100. Later doses were to be specified on the basis of the pharmacokinetic analysis of these cases. Paclitaxel was to be given at the conventional dose of 175 mg/m2 as a three hour infusion each three weeks, with standard premedications (dexamethasone, diphenhydramine, cimetidine).

Since March 5, 1996, we have now treated nine patients, as shown in Table 1. The major findings have been a significant occurance of moderate to severe skin toxicity: an erythematous follicular eruption of the face, trunk, and upper extremities of grade 2-3 in 4/8 evaluable patients. Several photographs of these reactions are appended within. Biopsy of these lesions has demonstrated superficial folliculitis, with adjacent edema and mixed neutrophil and eosinophil, or pure neutrophil-rich inflammatory cell infiltrate with scattered histiocytes. Immunohistochemistry for EGFR in these skin biopsies revealed normal expression within keratinocytes. Of eight patients evaluable for response, two have shown minor tumor regression, but one of these had to discontinue treatment because of dermatologic toxicity.

These data indicate synergistic biologic activity between HC Mab 225 and paclitaxel, but in the skin. We cannot yet assess if this synergy extends to the tumor, because the toxicity observed has precluded adequate evaluation, both in terms of number of patients accrued and duration of follow-up. However, no early indications of synergistic benefit have been observed. Because of these results, we are in the process of modifying the clinical protocol. Based on the hypothesis that the peak serum level of paclitaxel may contribute to the toxicity, we will reassess the Mab given weekly with a schedule of paclitaxel weekly at 80 mg/m2 as a one hour infusion (with standard premedications). In patients with ovarian carcinoma we have determined that this dose and schedule of paclitaxel is safe and effective, giving a response rate of about 30% in patients whose tumors have

previously demonstrated resistance to standard doses and schedules (8). We have initiated a trial of weekly one-hour paclitaxel (alone, without HC Mab 225) in patients with stage IV breast cancer, and so far have observed responses in 40% of cases. Hence, we will be combining HC Mab with an active regimen of paclitaxel but with one that achieves lower peak plasma levels because of the lower total dose per administration. Should this regimen fail to avoid unacceptable toxicity, we may combine HC Mab 225 with another schedule of paclitaxel, the 96-hour continuous infusion at 35 mg/m2/24h (repeated every three weeks). We have published that this dose and schedule produces responses in 27% of patients with breast cancer refractory to shorter taxane infusions, at peak plasma concentrations that are lower than achieved with bolus administration (9). Should that fail to avoid unacceptable toxicity, we plan to declare that the combination of paclitaxel with HC Mab is not feasible in humans, and initiate the study of the combination of doxorubicin and HC Mab 225 as outlined in the grant proposal.

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Table 1. Results of HC Mab 225 plus Paclitaxel in Stage IV Breast Cancer

#	EGFR	Dose	Response	Off-Study	Skin Toxicity (worst grade)
1	(+)	50/50	MR	PD 3 cycles	0
2	(+)	50/50	MR	SD 3 cycles	2
3	(+)	50/50	PD	PD 3 cycles	1
4	(-)	100/100	PD	PD 1 cycle	0
5	(+)	100/100	PD	PD during 1	2*
6	(-)	100/100	PD	PD after 1	3*
7	(-)	100/100	SD	PD after 1	1
8	(-)	100/100	SD	TOX	3*
9	(+)	100/100	SD	On Study	1

SD = Stable Disease
MR = Minor Reponse
PD = Progressive Disease
* = Skin Biopsy Obtained

APPENDIX